REDUCTION OF ACRYLAMIDE FORMATION IN COOKED STARCHY FOODS

Cross-Reference to Related Application

[0001] This application relies for priority on U.S. Provisional Application 60/424,151, filed November 6, 2002.

BACKGROUND OF THE INVENTION

(1) Field of the Invention

[0002] This invention relates to a process for the reduction of acrylamide formation in starchy foods when cooked at high temperature, particularly when they are baked or fried. This invention particularly addresses the problem of acrylamide formation in cooked starchy foods, generally baked or fried at temperatures above 120°C, particularly those selected from the group consisting of chips, tortilla chips, pretzels, crackers, backed goods, fried breads, processed cereals, and French fries. The process of this invention uses microbial cell fermentation to reduce acrylamide precursors (comprising mono- and di-saccharides and others) found in starchy foods prior to cooking. In particular, the present invention relates to a method wherein an uncooked starchy food product is treated with fermentative food grade bacteria and/or yeast under controlled pH and temperatures in the presence of growth stimulants comprising yeast extract and neutralizing

agents comprising alkali metal hydroxide (Na or K) or food grade acid (citric, lactic, or hydrochloric).

(2) Description of the Prior Art:

[0003] The use of acid producing bacterial cultures for food fermentations is well known. In general, the foods are preserved and a flavor is imparted to the food by the acid. The cultures are used for cheese, sausage, cottage cheese, yogurt and the like. Lactic acid is a primary metabolic product and is derived from sugars in the food. The cultures are sold commercially by multiple companies in lyophilized or frozen form. There is less lag time in beginning the fermentation in using the frozen cultures, which are thawed before use, and they are generally preferred. The concentrates usually contain 10^{10} to 10^{12} CFU per ml or gram of active bacteria.

[0004] The use of yeast in food fermentations is also well known. The yeasts are used in baked goods and beer. Generally the cultures are sold in packets which are refrigerated before use in order to preserve the yeasts.

[0005] The U.S. Environmental Protection Agency (EPA) classifies acrylamide, a colorless, crystalline solid, as a medium hazard probable human carcinogen. According to the International Agency for Research on Cancer, acrylamide induces gene mutations and has been found in animal tests to cause benign and malignant stomach tumors. It is also known to cause damage to the central and peripheral nervous system. Acrylamide became a

source of debate in April 2002, (Science 297 27 (2002)) when Swedish researchers announced that the chemical was present at high levels in starch-based foods that were fried or baked at temperatures higher than 120°C. They found that acrylamide levels in potato chips, French fries, bread and processed cereals are often hundreds of times higher than the maximum level, 0.5 µg per liter, considered safe for drinking water by World Health Organization (WHO) and the U.S.EPA. The highest levels were found in potato chips (a median of 1,200 ppb) and French fries (450 ppb). Subsequent studies in Norway, Switzerland, the U.K., and the U.S. confirmed the results. Meanwhile, the Food and Drug Administration (FDA) and other world health agencies are drafting action plans to assess human dietary exposures to acrylamide, gathering information about its toxicology, and asking the food industry to develop techniques for reducing its formation in food.

[0006] The detailed reaction mechanism for the formation of acrylamide involves the reaction of a sugar such as glucose and asparagine. Potatoes in particular contain significant amounts of free asparagine.

[0007] Acrylamide was not found in boiled or uncooked starchy foods; therefore, it is a by-product of high-temperature cooking processes. Different cooking times and temperatures could give rise to the variability of acrylamide levels in foods. Frying foods such as French fries or chips at low temperatures (less than 120°C) might reduce the formation of acrylamide, but this is very detrimental to the texture and flavor. Frying

foods at regular frying temperatures (176 - 190°C) for a very short period of time might reduce the formation of acrylamide, but these conditions are also very detrimental to the flavor and texture. Moreover, all food must be cooked properly to destroy food poisoning bacteria. Therefore, the prior art has recognized the need for methods to reduce the formation of acrylamide in cooked starch foods that are safe and not detrimental to the flavor and texture.

OBJECTS

[0008] It is an object of the present invention to provide a method for the reduction of acrylamide formation in cooked starchy foods without altering their regular cooking process parameters (temperature and time). Further, it is an object of the present invention to reduce acrylamide precursors found in starchy foods prior to cooking using microbial cell fermentation. Further still, it is an object of the present invention to provide methods that are inexpensive to perform, which preserve the flavor and texture of the cooked starchy food and which can be easily scaled-up to large volumes using conventionally available equipment. These and other objects will become increasingly apparent by reference to the following discussion and drawings.

SUMMARY OF THE INVENTION

[0009] The present invention relates to a process for reducing acrylamide production in a cooked, starch based

processed food which comprises: fermenting the processed food before cooking in an agitated aqueous medium with a microorganism used for food fermentations so as to ferment sugars in the food sufficiently to reduce the acrylamide production upon cooking; and cooking the food, wherein the fermented and cooked food contains less acrylamide than without the fermentations. The aqueous medium can comprise an added sugar. The aqueous medium can also comprise an added amino acid source.

[0010] Preferably the aqueous medium is at a temperature between about 10 and 40°C. Preferably the pH of the aqueous medium is between 4 and 8 during the fermentation.

[0011] The food can be fried or baked. Preferably the food is selected from the group consisting of potato chips, tortilla chips, pretzels, crackers, baked goods, fried breads, processed cereals and French fries. For French fries and potato chips the aqueous medium is in a reaction vessel and the aqueous medium is recirculated into and out of the vessel while retaining the food in the vessel.

[0012] The microorganism is a yeast or a bacterium which is food grade. Mixtures of the microorganisms can be used such as a yeast and a bacterium. Preferably the microorganism is a lactic acid producing microorganism. The microorganisms can be recycled between batches of the food which are processed. Preferably prior to fermenting, a pH of the aqueous medium is adjusted to reduce the acrylamide production. Preferably the food, such as French fries, is washed with water prior to the

cooking to remove residues of the fermentation. Preferably at the end of the fermenting the aqueous medium has a pH between about 4 and 5. After the fermenting the food can be dried to remove moisture. This is important with meals such as tortilla chips, cereal and cornmeals.

[0013] Thus the present invention relates to a process for the reduction of acrylamide formation in starchy foods cooked at high temperature by removing the acrylamide precursors present in these foods prior to cooking. This process renders acrylamide precursors unavailable (non-reactants) to the acrylamide synthesis reaction that occurs at high temperature (>120°C) and it is based on using microbial cell fermentation. The process comprises the steps of reacting a mixture of uncooked starchy foods with distilled water (or other purified water) containing yeast extract and/or food grade bacteria, such as lactic acid bacteria and others. The bacteria can be selected from the genera consisting of, but not limited to, *Streptococcus* spp., *Aerobacter* spp. *Escherichia* spp, *Leuconostoc* spp. The food grade yeast can be selected from the genera consisting of, but not limited to, *Saccharomyces* sp., *Torulopsis* spp., *Candida* spp. and mixtures thereof at optimum pH and temperature with mixing as long as required in order to achieve the highest reduction of acrylamide in these foods when cooked at high temperature.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1 is a flow diagram showing a preferred

microbial fermentation process for the removal of acrylamide precursors in raw potato slices prior to cooking. The process particularly includes reacting 500 ml distilled water containing 0.5 g dry yeast extract and 2.5 g active dry yeast (*Saccharomyces cerevisiae*) or 2.5×10^{12} bacterial cells (*Streptococcus lactis* or *thermophilus*) with 100 g raw potato slices for 2.5 hr at pH 6 and temperature 30°C.

[0015] Figure 2 is a schematic cross-sectional view of a fermenter 10 equipped with a mixing pump (not shown) illustrating the construction and facilities for control.

[0016] Figure 3 is a schematic cross-sectional view of a fermenter 30 equipped with a conventional mixer and impeller illustrating the construction and facilities for control.

[0017] Figure 4 is a flow diagram showing a microbial fermentation process for the removal of acrylamide precursors in fabricated potato chip mix with no sugar added prior to cooking. The process includes reacting 500 ml distilled water containing 0.5 g dry yeast extract and 2.5 g active dry yeast (*Saccharomyces cerevisiae*) or 2.5×10^{12} bacterial cells (*Streptococcus lactis* or *thermophilus*) with 100 g fabricated potato chip mix for 2.5 hr at pH 4 and temperature 30°C.

[0018] Figure 5 is a flow diagram showing a microbial fermentation process for the removal of acrylamide precursors in processed cereal mix with no sugar added prior to cooking. The process includes reacting 500 ml distilled water containing 0.5 g dry yeast extract and

2.5 g active dry yeast (*Saccharomyces cerevisiae*) or 2.5 x 10^{12} bacterial cells (*Streptococcus lactis* or *thermophilus*) with 100 g processed cereal mix for 2.5 hr at pH 4 and temperature 30°C.

[0019] Figure 6 is a flow diagram showing a microbial fermentation process for the removal of acrylamide precursors in corn tortilla chip masa prior to cooking. The process includes reacting 500 ml distilled water containing 0.5 g dry yeast extract and 2.5 g active dry yeast (*Saccharomyces cerevisiae*) or 2.5 x 10^{12} bacterial cells (*Streptococcus lactis* or *thermophilus*) with 100 g corn tortilla chip masa for 2.5 hr at pH 4 and temperature 30°C.

DESCRIPTION OF PREFERRED EMBODIMENTS

[0020] The method for removal of acrylamide precursors in starchy foods prior to cooking largely prevents the formation of acrylamide in these foods when cooked at high temperatures.

[0021] A preferred process for removal of acrylamide precursors in raw potato slices used to make potato chips is outlined in Figure 1 using the fermenter illustrated in Figure 2. The fermenter 10 includes strainer 11, aqueous solution inlet 12, cooling water jacket 13, cooling water inlet 14, steam inlet 16, harvest for condensed steam 16, aqueous solution outlet 17, sterile sealer 18, aqueous medium 19, cooling water outlet 20, exhaust 21, neutralizing agent reservoir 22, pH-meter 23, conventional pump 24, and potato slices 25. The process uses microbial cells consisting of

Saccharomyces cerevisiae or *Streptococcus lactis* (also called *Lactococcus lactis)* or *Streptococcus thermophilus*.

Materials and Methods:

[0022] Potato tubers, an experimental variety "Wisconsin 123", were obtained from The Michigan Potato Industry Commission. The sugar profile and free amino acids contents are reported in Table 1.

[0023] The sugar profile was analyzed using the Official Methods of Analysis of AOAC INTERNATIONAL (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 982.14. (Modified).

[0024] The free amino acids were analyzed using AOAC International, 982.30 'Protein Efficiency Ratio' (modified), Official Methods of Analysis, (ed.) Patricia Cunniff, Sixteenth Ed., Vol. 2, AOAC International: Arlington, VA (1995).

[0025] Acrylamide was analyzed by Liquid Chromatography-Mass Spectrograph (LCMS), United States Food and Drug Administration, Center for Food Safety and Applied Nutrition Office of Plant and Dairy Foods and Beverages, "Detection and Quantitation of Acrylamide in Foods" (2002).

TABLE 1
Sugar Profile and Free Amino Acids Contents of the Raw and Peeled Potatoes* Used in This Invention

	Gram/100 grams
Sugar Profile	
Fructose by HPLC	<0.1
Glucose by HPLC	<0.1
Sucrose by HPLC	<0.1
Maltose by HPLC	<0.1
Lactose by HPLC	<0.1
Free Amino Acids	
Aspartic Acid	0.021
Glutamic Acid	0.063
Proline	0.015
Glycine	0.004
Alanine	0.027
Cystine	<0.001
Valine	0.031
Methionine	0.014
Isoleucine	0.012
Leucine	0.011
Tyrosine	0.024
Phenyalanine	0.017
Histidine	0.023
Lysine	0.025
Arginine	0.142
Asparagine	0.411

*Experimental variety: "Wisconsin 123"

The potatoes had a significant asparagine content.

EXAMPLE 1

[0026] A fermentation medium was prepared consisting of 500 ml distilled water heated to 30°C and 0.5 g dry yeast extract. The 2.5 g active dry yeast (*Saccharomyces cerevisiae*) or 2.5×10^{12} bacterial cells (*Streptococcus lactis* or *thermophilus*) were added. A conventional pump was used with the fermenter which circulated the aqueous fermentation medium into and out of the mixing tank in a loop form to maintain a uniform medium. The washed raw potato slices (100 g) were added in a very quick succession. The aqueous medium was pumped out of the mixing tank through a strainer which prevented the potato slices from going through the pump to avoid any physical damages to the slices caused by the pump. The pH was adjusted to 6 using a pH-meter and neutralizing agent. The temperature during processing was maintained at 30°C using steam. The processing time of the mixture was 2.5 hr. The sugars available were reduced by the fermentation as was the acrylamide factor upon frying of the potato slices. Generally the fermentation strives for complete inhibition of acrylamide formation in the fried chips.

EXAMPLE 2

[0027] The effect of varying microbial cell concentrations on the reduction of acrylamide formation in fried potato chips and the removal of acrylamide precursors (mono- and di-saccharides and others) prior to cooking was determined using the experimental conditions outlined in Figures 1 and 2, and reported in

the following experiments in Tables 2 and 3.

TABLE 2

Effect of active dry yeast (*Saccharomyces cerevisiae*) concentrations

Yeast (g)	pH	Temp. (°C)	Yeast Extract (g)	Substrate (g)	Time (hr)	Acrylamide Reduction (%)	Mono & di-saccharides (%)
0.5	6	30	0.5	100	2.5	20	<0.1
1.5	6	30	0.5	100	2.5	24	<0.1
2.5	6	30	0.5	100	2.5	19	<0.1
5	6	30	0.5	100	2.5	21	<0.1
10	6	30	0.5	100	2.5	16	<0.1

The data suggest that the medium is saturated with yeast cells at the tested concentrations.

TABLE 3

Effect of bacterial cells (*Streptococcus thermophilus*) concentrations

Bacterial (count) CFU/ml	pH	Temp. (°C)	Yeast Extract (g)	Substrate (g)	Time (hr)	Acrylamide* Reduction (%)	Mono & di-saccharides (%)
$10x10^8$	6	30	0.5	100	2.5	40	<0.1
$30x10^8$	6	30	0.5	100	2.5	51	<0.1
$50x10^8$	6	30	0.5	100	2.5	68	<0.1
$10x10^9$	6	30	0.5	100	2.5	70	<0.1
$20x10^9$	6	30	0.5	100	2.5	62	<0.1

*In the aqueous medium the data shows greater acrylamide reduction with a higher concentration of the bacteria.

EXAMPLE 3

[0028] Temperature is a factor in a fermentation

reaction due to its ability to: (1) change the rate of the reaction, and (2) inactivate the microbial cells. The effect of the reaction temperature on reduction of acrylamide formation in fried potato chips and the removal of acrylamide precursors (mono- and di-saccharides and others) prior to cooking was determined according to the experimental conditions outlined in Figures 1 and 2, and reported in the following experiments in Tables 4 and 5.

TABLE 4

Effect of incubation temperature using active dry yeast (*Saccharomyces cerevisiae*)

Yeast (g)	pH	Temp. (°C)	Yeast Extract (g)	Substrate (g)	Time (hr)	Acrylamide Reduction* (%)	Mono & Di-saccharides (%)
2.5	6	25	0.5	100	2.5	21	<0.1
2.5	6	30	0.5	100	2.5	27	<0.1
2.5	6	35	0.5	100	2.5	25	<0.1

*The temperature did not change the acrylamide reduction.

TABLE 5

Effect of incubation temperature using bacterial cells (*Streptococcus thermophilus*)

Bacterial (count) CFU/ml	pH	Temp. (°C)	Yeast Extract (g)	Substrate (g)	Time (hr)	Acrylamide Reduction* (%)	Mono & di-saccharides (%)
$5x10^9$	6	25	0.5	100	2.5	51	<0.1
$5x10^9$	6	30	0.5	100	2.5	59	<0.1
$5x10^9$	6	35	0.5	100	2.5	63	<0.1

*The high temperature increased the acrylamide reduction.

EXAMPLE 4

[0029] The fermentation process can be continued as long as required in order to achieve the highest reduction of acrylamide formation in fried chips. The time involved in the reaction is dependent upon the level of microbial cells used. Microbial cell levels compatible with good economic processing, involve shorter processing times. The effect of incubating time on reduction of acrylamide formation in fried potato chips and the removal of acrylamide precursors (mono- and di-saccharides and others) prior to cooking was determined according to the experimental conditions outlined in Figures 1 and 2, and reported in the following experiments in Tables 6 and 7.

TABLE 6

Effect of incubation time using active dry yeast (*Saccharomyces cerevisiae*)

Yeast (g)	pH	Temp. (°C)	Yeast Extract (g)	Substrate (g)	Time (hr)	Acrylamide Reduction* (%)	Mono & di-saccharides (%)
2.5	6	30	0.5	100	0.5	4	<0.1
2.5	6	30	0.5	100	1.0	10	<0.1
2.5	6	30	0.5	100	2.5	21	<0.1
2.5	6	30	0.5	100	4.0	28	<0.1
2.5	6	30	0.5	100	6.0	22	<0.1

*Longer times increased acrylamide reduction.

TABLE 7

Effect of incubation time using bacterial cells (*Streptococcus termophilus*)

Bacterial (count) CFU/ml	pH	Temp. (°C)	Yeast Extract (g)	Substrate (g)	Time (hr)	Acrylamide Reduction* (%)	Mono & di-saccharides (%)
$5x10^9$	6	30	0.5	100	0.5	20	<0.1
$5x10^9$	6	30	0.5	100	1.0	28	<0.1
$5x10^9$	6	30	0.5	100	2.5	61	<0.1
$5x10^9$	6	30	0.5	100	4.0	64	<0.1
$5x10^9$	6	30	0.5	100	6.0	55	<0.1

*Longer times increased acrylamide reduction

EXAMPLE 5

[0030] The rate of microbial fermentation reactions is directly affected by the pH of the fermentation medium. The effect of different pHs on the reduction of acrylamide formation in fried potato chips and the removal of acrylamide precursors (mono- and di-saccharides and others) prior to cooking was determined according to the experimental conditions outlined in Figures 1 and 2, and reported in the following experiments in Tables 8 and 9.

TABLE 8

Effect of pH using active dry yeast (*Saccharomyces cerevisiae*)

Yeast (g)	pH	Temp. (°C)	Yeast Extract (g)	Substrate (g)	Time (hr)	Acrylamide Reduction* (%)	Mono & di-saccharides (%)
2.5	4	30	0.5	100	2.5	74	<0.1
2.5	5	30	0.5	100	2.5	60	<0.1
2.5	6	30	0.5	100	2.5	23	<0.1
2.5	7	30	0.5	100	2.5	20	<0.1
2.5	8	30	0.5	100	2.5	25	<0.1

*pH had a marked effect on acrylamide reduction

TABLE 9

Effect of pH using bacterial cells (*Streptococcus thermophilus*)

Bacterial (count)	pH	Temp. (°C)	Yeast Extract (g)	Substrate (g)	Time (hr)	Acrylamide Reduction* (%)	Mono & di-saccharides (%)
$5x10^9$	4	30	0.5	100	2.5	81	<0.1
$5x10^9$	5	30	0.5	100	2.5	72	<0.1
$5x10^9$	6	30	0.5	100	2.5	64	<0.1
$5x10^9$	7	30	0.5	100	2.5	45	<0.1
$5x10^9$	8	30	0.5	100	2.5	31	<0.1

*pH had a marked effect on acrylamide reduction

[0031] At pH 4, the observed acrylamide reduction was higher than the ones observed at the optimal pH (6 or 7) for microbial growth. The higher acrylamide reduction at pH 4 indicates that a low pH has effect on the acrylamide reduction along with microbial fermentation.

EXAMPLE 6

[0032] The nature and the physical state of acrylamide precursors (substrate) containing foods have a major effect on the rate of the fermentation reaction. They affect the accessibility of the substrate to the reactions sites in the microbial cells. The effect of different forms of acrylamide precursor containing foods on the reduction of acrylamide formation in these foods when cooked at high temperature and the removal of these precursors (mono- and di-saccharides and others) from these foods prior to cooking was determined according to the experimental conditions outlined in Figures 4, 5, and 6 using the fermenter 30 described in Figure 3 as summarized in the following set of experiments. The fermenter 30 includes mixer 31, impeller 32, cooling water jacket 33, cooling water inlet 34, steam inlet 36, condensed steam harvest 36, cooling water outlet 37, exhaust 38, neutralizing agent reservoir 39, pH-meter 40, sterile seal 41 and reactants 42.

[0033] A fermentation medium was prepared containing 500 ml distilled water which was heated to 30°C and 0.5 g dry yeast extract. The 2.5 g active dry yeast (*Saccharomyces cerevisiae*) or 2.5×10^{12} bacterial cells (*Streptococcus lactis* or *thermophilus*) were added while mixing using a conventional mixer equipped with a shaft and impeller 32 as shown in Figure 3. The acrylamide precursor containing foods (100g) were added in a very quick succession. The pH was adjusted to 4 using a pH-meter and neutralizing agent. The temperature during processing was maintained at 30°C using steam. The

processing time of the mixture was 2.5 hr. The results are shown in Table 10.

TABLE 10

Effect of different forms of acrylamide precursors containing foods using bacterial cells (*Streptococcus thermophilus*)

Bacterial (Count)	pH	Temp. (°C)	Yeast Extract (g)	Foods	Time (hr)	Acrylamide Reduction* (%)	Mono & di-saccharides (%)
$5x10^9$	4	30	0.5	A(Fig.4)	2.5	81	-
$5x10^9$	4	30	0.5	B(Fig.5)	2.5	72	-
$5x10^9$	4	30	0.5	C(Fig.6)	2.5	66	-

A:fabricated potato chip mix, B: processed cereal mix, C: corn tortilla chip masa.

*There was a significant reduction of acrylamide.

[0034] While particular embodiments of the invention is illustrated and described, it will be obvious to those skilled in the art that various changes and modifications can be made without departing from the spirit and scope of this invention.